Amendments to the Specification

Please amend the paragraph on page 13, starting at line 17, to the following:

A "producer cell" is a cell that comprises a helper virus and a viral vector. The viral vector is employed to transduce a packaging cell to form a producer cell capable of assembly ~~if~~ of infectious vector particles. Examples of packaging cells include, but are not limited to the PE501, PA317, Ψ2, Ψ-AM, PA12, T19-14X, VT-19-17-H2, ΨCRE, ΨCRIP,GP+E-86, GP+envAM12, and DAN cell lines. The vector containing the foreign nucleotide sequence may transducer the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. The producer cells may then be directly administered, whereby the producer cells generate viral particles capable of transducing the recipient cells.

Please amend the paragraph on page 21, starting at line 30, to the following:

Applicants invention includes techniques and methods which disclose ~~the~~ for the first time the drastic effects helper virus methylation and the ability to identify, select for, and maximize the presence of active helper virus. Any number of ways of restricting methylation or of reducing the presence of methylated helper virus are also intended to be included herein, including but not limited to: treatment of vector producer cells with 5-aza-C, insertion of a demthylation fragment of murine Thy-1 in front of the 5′ long terminal repeat, ligation of an internal ribosome entry site with a selection marker so that drug selection would ensure promoter function, use of immune response selection, design of synthetic viral promoters to omit methylation sites, screening for other drugs which inhibit methylation, and even antisense inhibition to the human methylase gene which is known and readily accessible through sources such as Genbank.

Please amend the paragraph on page 32, starting at line 22, to the following:

Cell culture and transfection. Cell cultures were maintained in Dulbecco's modified Eagle medium (DMEM; GIBCO BRL, Life Technology Co., Gaithersburg, MD), 10% fetal calf serum with 5% CO_2, at 37°C. The subclones of LTKOSN.2 VPC were obtained by limiting dilution of parental LTKOSN.2 VPC onto two 96-well plates (Young, W.-B., supra). Helper virus and vector gene expression, DNA methylation status and vector production in these subclones have been previously characterized (Young, W.-B., supra). To rescue LTKOSN and ~~Δ~~ΔLTKOSN

vectors from pre-existing LTKOSN VPC subclones with methylated and silenced helper virus DNA, the subclones were transfected with pAM3-IRES-Zeo using Fugene 6TM transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN). To study the effects of host DNA methylation on retroviral helper virus without interference from chromosomal copies pPAM3 present in LTKOSN VPC, pAM3-IRES-Zeo plasmid was transfected into NIH3T3 *tk*$^-$ cells [American Type Culture Collection (ATCC) CRL1658] utilizing Fugene 6^{TM} transfection reagent. A mixed population pAM3-IRES-Zeo-transfected NIH3T3 *tk*$^-$ cells, termed AMIZ cells, was established. Prior to transfection, pAM3-IRES-Zeo plasmid was linearized by *BspHI* digestion and 6 to 10 μg of pAM3-IRES-Zeo was then transfected to each well in 6-well plates. Selection with Zeocin^{TM} (350 μg/ml, Invitrogen) began 48 hr after transfection and continued for at least two weeks. Transfection of LEIN vector into the AMIZ cell pool and GP+E86 packaging cells (Markowitz, D., supra) (kindly provided by Arthur Bank, Columbia University, New York, NY) was completed by DOTAP Liposomal Transfection Reagent (Roche Molecular Biohemicals) with 5 μg of LEIN plasmid for each well in 6-well plates. Selection with G418 (1 mg/ml; GIBCO) started 48 hr after transfection and continued for two weeks.

Please amend the paragraph on page 38, starting at line 3, to the following:

Retroviral superinfection is blocked by enhanced helper virus gene expression. The effect of Zeocin^{TM}~~TM~~ selection on AMIZ cells was analyzed by gene expression of pAM3-IRES-Zeo in AMIZ cells. Gene expression of pAM3-IRES-Zeo in AMIZ cells with constant Zeocin^{TM} selection showed a 2-fold increase compared to AMIZ cells without selection on Day 15 and at least 4-fold increase on Days 54 and 78 (Fig. 10). In contrast, pAM3-IRES-Zeo gene expression in AMIZ cells without Zeocin^{TM} selection declined over time (Fig. 10, lanes 3, 5 and 7). Continuous Zeocin^{TM} selection may have selected integration sites that are highly transcriptionally active and have less DNA methylation activity (Cedar, H. 1988. DNA methylation and gene activity. Cell. 53:3-4; Keshet, I., supra).

We directly determined whether decreased pAM3-IRES-Zeo gene expression reduced Env-receptor interference and increased vector superinfection. The susceptibility to superinfection was measured by exposing AMIZ cells from the above experiment to amphotropic LEIN vector supernatants followed by G418 selection. G418 resistant colony number obtained from AMIZ cells with continued ZeocinTM selection was reduced from 2.3 X 10^1 on Day 15 to no superinfection observed on Days 54 and 78 (Table 2). In contrast, G418 resistant colonies obtained from AMIZ cells without Zeocin^{TM} selection ranged from 1.2 X 10^3 to 5.6 X 10^3. These

results demonstrate that increased gene expression of helper virus correlates with reduced susceptibility to superinfection.

High level of vector production was maintained by Zeocin™~~TM~~ selection. Vector production was analyzed in this AMIZ cell pool by transfecting LEIN vector into AMIZ cells followed by G418 selection to establish a VPC for titer assay. Zeocin™ selection was temporally withdrawn from AMIZ cell culture during the first three weeks of G418 selection after transfection with LEIN vector. Titer obtained from this newly established uncloned population of AMIZ cells was 3.5 X 10^6 cfu/ml, which is 100-fold higher than the titer observed from a mixed population of PA317 transfected with LEIN vector (4 X 10^4 cfu/ml). In addition, AMIZ cells were transduced with LEIN vector collected from LEIN-transfected GP+E86 cells and an improved titer of 9 X 10^6 cfu/ml was obtained from a mixed cell population. To investigate whether selection with both Zeocin™ and G418 would adversely affect vector production, LEIN transfected AMIZ cells were evaluated 56 (8 passages) and 67 days (10 passages) after transfection. Titers obtained from AMIZ cells transfected with LEIN (3.5 X 10^6 cfu/ml on Day 0) and placed under continuous selection with Zeocin™ and G418, were 2 X 10^6 cfu/ml (Day 56) and 1.5 X 10^7 cfu/ml (Day 67). In contrast, titers obtained from the same AMIZ cells transfected with LEIN but not subjected to G418 and Zeocin™ selection only showed 2 X 10^4 and 4 X 10^4 cfu/ml on Day 56 and Day 67, respectively. The reduced titer correlated with a significant decrease of both helper virus and vector gene expression when time points with and without selection were compared (Fig. 11). No significant increase of titer or helper virus gene expression was observed when the 17% DNA methylation present on Day 0 was further reduced to 0% DNA methylation by Day 56 after selection. This suggests a threshold effect as we previously observed in cloned VPC (Young, W.-B., supra). Substantial decreases of vector production, helper virus gene expression and Env-receptor interference was only observed once at least 60% methylation occurred of the helper virus 5'LTR.

DNA methylation status of 5'LTRs of helper virus and vector were significantly increased in AMIZ cells transfected with LEIN vector and cultured without either G418 or Zeocin™ selection (Fig. 12). This increased methylation corresponded to above decreased vector titer and significantly reduced gene expressions of helper virus and vector (Fig. 11). The DNA methylation of helper virus 5'LTR increased from 17% (Day 0) to 30% and 36% by Days 56 and 67, respectively. The average DNA methylation rate of helper virus 5'LTR in AMIZ cells transfected with LEIN was estimated as low as 0.3% of the cell population per day during 67 days of continuous cell culture. In contrast, DNA methylation was not detected in AMIZ cells transfected with LEIN vector and placed under continuous G418 and Zeocin™~~TM~~ selection. No detectable DNA methylation occurred in the LEIN vector on Day 0 (Fig. 12C, lanes 3 and 4) while the 5'LTR helper virus showed 17% DNA methylation (Fig. 12B, lanes 3 and 4). This may

be secondary to the timing of G418 and ZeocinTM selection. AMIZ cells transfected with LEIN vector were placed under G418 for three weeks to select for LEIN-positive population and Zeocin™ selection was not applied until Day 0 in the experiment.

Please amend the paragraph starting on page 41, starting at line 28, to the following:

The selection of transfected cells (AMIZ cells) with Zeocin™~~TM~~ to maintain pAM3-IRES-Zeo gene expression eliminated DNA methylation from AMIZ cells and may also select cells with pAM3-IRES-Zeo helper virus integrated in optimal and active chromosomal regions. Ratios of pAM3-IRES-Zeo gene expression in selected AMIZ cells compared to non-selected AMIZ cells were about 2:1 on Day 15 and at least 4:1 on Days 54 and 78 (Fig. 10), while helper virus showed only 12%, 19% and 61% of DNA methylation, respectively (Fig. 9). Similar results were also observed in AMIZ cells transfected with LEIN vector. Cells under continuous selection showed no detectable DNA methylation of the 5'LTR, but 30% (Day 56) and 36% (Day 67) of DNA methylation was detected in cells without selection (Fig. 12). LEIN-transfected AMIZ cells under continuous selection had vector titer of 1.5×10^7 cfu/ml on Day 67, compared to 4×10^4 cfu/ml on Day 67, in cells without selection. This 1000 fold difference in titer production probably reflects the fact that structural proteins of viruses function as multimers (Hunter, E., supra). The formation of multimers occurs in a sigmoid dose-response fashion, rather than a linear dose-response to protein concentration that correlates more directly with helper virus gene expression and DNA methylation. The effect of host DNA methylation on helper virus 5'LTR is therefore amplified by transcription, viral assembling and then vector production.